

17
REMARKS

Favorable reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-99 are in this case. Claims 21-50 and 71-99 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 1-20 and 51-70 have been rejected. Claims 1-8, 10, 20, 51-57, 60 and 70 have now been amended.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 1-20 and 51-70 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiner's rejections are respectfully traversed. Claims 1 and 51 have now been amended according to Examiner's suggestion, rendering moot the Examiner's rejection.

35 U.S.C. § 103(a) Rejections

The Examiner has rejected claims 1-20 and 51-70 under 35 U.S.C. § 103(a) as being unpatentable over Naughton et al. (U.S. Pat. No. 5,541,107) in view of Sussman et al. (U.S. Pat. No. 5,266,476) and Stephanopoulos et al. (U.S. Pat. No. 5,510,262).

The Examiner finds the 37 CFR Declaration filed with the previous response unpersuasive. Specifically, the Examiner states that the declaration shows that a flow system bioreactor is needed to support 3-D stroma cells to allow HSC expansion when compared with a static system without flow. The Examiner states that Naughton et al. can use continuous flow of culture medium and does not have to use static system. The Examiner further states that use of the fibrous matrix of Sussman et al. in a flow through system for cell culturing would have been obvious since it can be utilized in such a system and since it will provide expected advantages. Finally, the Examiner contends that when using continuous flow of medium as disclosed by Naughton et al., a difference in results as shown by the declaration would not be obtained. Continuous medium flow is obviously going to provide more nutrients to the cells and better removal of waste products, and result in more cell growth than when using static

conditions.

Following an interview with the Examiner conducted on September 14, 2004, it was mutually agreed that amending the claims to include the limitations set forth herein will be favorably considered by the Examiner.

The ex vivo expansion of hemopoietic stem cells (HSCs) is the subject of intense commercial and academic interest due to the potential of HSCs to be a renewable source of material for cellular therapeutics. Unfortunately, because methodologies have not yet been successfully developed to grow clinically relevant numbers of HSCs (or their derivatives) consistently, the potential of this technology is limited. The challenge of producing clinically useful amounts of HSCs is the subject of the HSC appendix which is enclosed herewith.

The present invention relates to a novel approach, which can be used for growing and expanding non-differentiated stem cells. As is evident from the rejections presented by the Examiner, such an approach has not been described or suggested in the prior art. Although the Examiner contends that the combination of prior art references supporting the 103 rejection would have motivated the ordinary skilled artisan to make and use the present invention, several lines of evidence support the contrary, i.e., that the prior art teaches away from the present invention.

Applicant asserts that the combination of patents described by the Examiner has been available for years (i.e., from 1996) however despite the advantages claimed by the Examiner no one has combined these teachings to arrive at the present invention. Given the commercial and scientific implications of the present invention in the growing field of stem cell technology and tissue engineering, one would expect that an ordinary skilled artisan would make the present invention well before the filing date of the instant application if indeed, as claimed by the Examiner, these references provide the teachings and motivation.

But that is clearly not the case, in fact, one of the patents referenced by the Examiner is owned by Advanced Tissue Sciences, Inc. (La Jolla, CA), a leading tissue engineering company engaged in the development of living human tissue products for therapeutic applications (e.g., TranscyteTM, Dermagraft® and NeoCyteTM). If indeed these prior art references are capable of motivating the ordinary skilled artisan to make

and use the present invention, they failed to motivate a company, which could greatly benefit from such an approach in stem cell growth, which would undoubtedly enhance their capabilities to fabricate their products.

In view of the above, it is Applicant strong opinion that the state of the art at the date of filing of the present invention in fact would not motivate the skilled artisan to make the present invention.

A literature analysis conducted by the Applicant revealed that the state of the art at the time of filing not only would not motivate the ordinary skilled artisan but in fact teaches away from the present invention (i.e., growth and expansion of non-differentiated hematopoietic stem cells under flow conditions).

Prior art studies such as those referenced below clearly teach that static conditions promote growth of non-differentiated cells and that flow conditions promote cell differentiation, which is often accompanied by apoptosis and not by proliferation (expansion). Clearly, the skilled artisan would not be motivated to use flow conditions for growing and expanding non-differentiated hematopoietic stem cells, as taught by the present invention.

The following summarizes scientific publications as well as patents that compare the effect of static conditions and flow conditions (shear stress) on the growth and differentiation of various cell types.

(i) Flow conditions enhanced osteogenic differentiation of human bone marrow stromal cells on 3-D partially demineralized bone scaffolds in vitro as compared to static conditions [Mauney (2004) *Calcif. Tissue Int.* 74(5):458-68].

(ii) Flow conditions enhanced osteoblast proliferation and differentiation as compared with static conditions [van den Dolder (2003) *J. Biomed. Mater. Res.* 64A(2):235-41].

(iii) Similarly, Gomes and co-workers have shown that flow perfusion culture enhances the osteogenic differentiation of marrow stromal cells as compared to a static culture [Gomes (2003) *J. Biomed. Mater. Res.* 67A(1):87-95.

(iv) Flow conditions were also shown to increase proliferation and differentiation of embryonic stem cells and to result in the formation of embryoid bodies [Gerecht-Nir (2004) *Biotechnol. Bioeng.* 86(5):493-502].

In addition, significant efforts have been put on investigating the effect of shear stress arising from flow culturing conditions on cell differentiation.

For example, Masatugu and co-workers have shown that flow conditions (obtained while using parallel plate-type flow chamber), induce shear stress which inhibits proliferation of vascular smooth muscle cells as compared with static conditions [Masatsugu (2003) *Regul. Pept.* 111(1-3):13-9].

Endothelial cell-derived endothelin-1 (ET-1) contributes to intimal hyperplasia in re-endothelialized segments of vascular grafts and angioplasty sites. Sharefkin and co-workers and Morawietz et al. tested the effects of static conditions versus high laminar shear stress conditions (flow) on endothelial cell differentiation as monitored by the release of the mitogenic factor ET-1 from cultured human endothelial cells. It was clearly shown that shear stress significantly reduces ET-1 secretion as compared to static conditions, indicating that flow conditions inhibit proliferation of endothelial cells and stromal cells from intimal hyperplasia [Sharefkin (1991) *J. Vasc. Surg.* 14(1):1-9; Morawietz et al. (2000) *J. Physiol.* 525 Pt 3:761-70].

Rhoads and co-workers have shown the fluid flow induces aortic smooth muscle cell differentiations as indicated by the release of fibroblast growth factor 2 [Rhoads (2000) *Arterioscler. Thromb. Vasc. Biol.* 20(2):416-21].

Shear stress is also known to increase apoptosis of culture cells. For example, Jessup and co-workers have shown that culturing of the poorly differentiated colorectal carcinoma cells, MIP-101, under static conditions (monolayers on Teflon-coated non-adherent surfaces) induced the highest rates of proliferation and lowest apoptosis as compared to flow cultures [Jessup (2000) *In Vitro Cell Dev. Biol. Anim.* 36(6):367-73].

As mentioned, it is static cultures, which were shown to support growth and expansion of non-differentiated hematopoietic stem cells as described in U.S. Pat. No. 6,642,049, while continuous flow conditions significantly inhibited growth while inducing differentiation of these cells (see U.S. Pat. Nos. 5,437,994, 5,647,750 and 5,922,597).

The use of static cultures was shown to be advantageous for other reasons as well. For example, the effective stiffness of cellular scaffolds is much higher under

static conditions than under flow conditions [see Engelmayr (2003) *Biomaterials* 24(14):2523-32].

In view of the above, it is Applicant's strong opinion that the prior art would not motivate one of ordinary skill in the art to use flow conditions to expand non-differentiated hematopoietic stem cells, as indeed is evident from the fact that although the prior art existed several years prior to filing of the instant application, no one described or even suggested use of flow conditions for the growth and expansion of non-differentiated hematopoietic stem cells.

Evidence for non-differentiated phenotype of expanded HSCs grown under the culturing conditions of the present invention is provided in the attached results appendix and in the Examples section of the instant application (see e.g., Figures 2-4).

Applicant further asserts that the commercial response to the invention further supports the non-obvious nature of the present invention, since it provides objective evidence of how the invention is viewed in the marketplace by those directly interested in the product. See *Arkie Lures, Inc. v. Gene Lanrew Tackle, Inc.* 119 F. 3d 953, 957 (Fed. Cir. 1997); and *Demaco*, 851 F. 2d at 1391.

In the instant case, when Applicant's invention was licensed to A.I. Software, Inc., and a public announcement thereof was made on May 5, 2003, investors immediately recognized the likelihood of commercial success of the licensed technology. A copy of the license agreement and the public announcement thereof is attached herewith. As evidenced by the price action of the stock of the licensing company and assignee of the present invention, Pluristem Life Systems Inc., attached herewith, the stock price of this publicly traded company exploded from 30 cents on April 21, 2003, when investors heard of these ongoing negotiations, to \$2.40 on May 19, 2004, an eightfold appreciation in price in under a month. The public immediately recognized that the value of this invention was going to result in substantial increase in income and profits to the company. Applicant has therefore introduced evidence and proof of the commercial success attributable to the instant invention and withdrawal of the rejection is deemed to be in order.

The Examiner further states that while the declaration describes the flow reactor apparatus in detail, it does not describe in detail the static reactor used. The Examiner concludes that the type of static reactor used would influence the difference in results obtained.

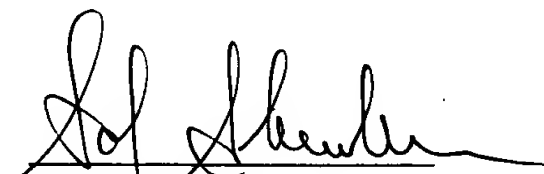
Applicant clarifies that the static conditions used in the experiments described in the Appendix, were applied by placing the substrate on which the stromal cells were seeded in a petri dish (to mimic the conditions described by Naughton and co-workers). The Appendix attached herewith has been amended to include the missing technical data. (i.e., petri dish details including size, vendor, city state etc.).

Finally the Examiner states that the flow reactor used contained four parallel units as shown by Figure 1. However, the reactor of the claims can differ substantially from that of Figure 1 and would not have to produce results when using the reactor of the figure.

Applicant notes that Figure 1 illustrates only one configuration of the plug-flow bioreactor of claim 1 and 51 and thus the components shown in Figure 1 are not specifically described in claim 1 and 51. It is appreciated that the plug flow bioreactor of the claimed invention can have many configurations as long as expanding/maintaining the undifferentiated hematopoietic stem cells is achieved.

In view of the above amendments and remarks, it is respectfully submitted that claims 1-20 and 51-70 are now in condition for allowance. An early Notice of Allowance is respectfully and earnestly solicited.

Respectfully submitted,



Sol Sheinbein

Registration No. 25,457

Date: October 9, 2004.

Enc.

"Results" Appendix

“HSC” Appendix

Declaration by Dr. Shai Meretski

Public announcement of the license agreement

Three-month extension fees